POLLEN WALLS AS ADAPTIVE SYSTEMS[1]

J. HESLOP-HARRISON[2,3]

WALL STRUCTURE AND THE MANY DIMENSIONS OF ADAPTATION

The structural variation of angiosperm pollen is primarily related to the wall, for the male gametophyte itself is simple in organization and more or less uniform in character throughout the group. The division of the wall into an outer exine and an inner intine is clearly a fundamental feature, as it is in the pollen grains and spores of other vascular groups, but the relative development of the strata differs widely among the families. The exine, usually the more structurally complex of the wall layers, is strikingly diverse in the types of wall sculpturing it can express, as well as in the numbers, distribution, and architecture of apertures and internal cavities. Faced with such variation, the first impulse is assuredly to attempt to bring some order and understanding by classification and ordination, and this has been the aim of palynologists since the days of von Mohl, a century-and-a-half ago. As this present symposium has shown, the task is by no means complete; but what has been achieved is already impressive, not least in the contribution pollen taxonomy has made to the advance of angiosperm taxonomy in general. If one's inclination is to ask for explanations, however, classification is not enough. The instinct is to seek for physiological meaning in the pollen grain wall—to attempt to understand what functions the structural features fulfill in the general biology of plants, and to search for some comprehension of the variation in form in evolutionary terms, seeking for evidence of adaptive diversification.

The essential function of the pollen grain and the tube that emerges from it is, of course, to deliver a pair of gametes to the embryo sac. The haploid male gametophyte with its single vegetative cell is structurally simple, but the attainment of its functional objective, the double fertilization, demands considerable physiological sophistication. This is expressed in a whole sequence of interlocking adaptations associated with dispersal, interaction with the stigma and style, nutrition, growth, and target finding. We now know that in the journey between the anther and the receptive stigma a major role is played by the pollen grain wall, which is concerned not only with protection and dispersal, but with the hydrodynamics of the gametophyte within it, and also in various subtle ways in the interactions on the stigma. The wall, in fact, serves many functions; and this must mean that in the course of angiosperm evolution it has been the target of many kinds of selective pressure. To it, as to any adaptive structure, a familiar truism must apply, namely, that the result of evolution under manifold selective forces must in each of the surviving lineages represent some sort of a compro-

---

[1] Text of the Evening Address given on the occasion of the Twenty-fifth Annual Systematics Symposium.

[2] Department of Botany, University of Massachusetts, Amherst, Massachusetts.

[3] Present address: Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, United Kingdom.

ANN. MISSOURI BOT. GARD. 66: 813–829. 1979.

0026-6493/79/0813–0829/$01.85/0

mise—a compromise that has been proved successful by the very fact of survival, but which nevertheless may not be optimal for each individual function.

Undoubtedly this helps us to understand some of the diversity encountered among the pollens of living angiosperms, and the more so when another truism of biological adaptation is taken into account, namely, that the same functional end can usually be attained by different morphological or physiological devices. The implication of this is that the compromise reached in the pollen walls of one group may be different from that attained in another. All are successful in some degree, but none is likely to be perfect. And to this we may add the further probability: that each may have elements not readily interpreted in adaptive terms at all, being related to lineage and the evolutionary events of a remote past, or being simply the products of fortuitous change.

I make these comments before turning to my main theme to avoid possible misunderstandings. Serious difficulties *do* arise in trying to interpret the intimate details of pollen structure in an adaptive sense, and one must be wary of carrying zeal for such interpretation too far. Such details do not *have* to be adaptive at all; or do not have to be adaptive in present circumstances. After all, there is no reason why structural idosyncracies not subject to intense selective pressures should not be conserved while other features undergo evolutionary change. It has long been supposed that such conserved characteristics are not only valuable as taxonomic criteria but offer good guides to phylogenetic affinity—an article of faith with those concerned with pollen phylogeny, just as it is with those whose interest embraces the whole organism. But the overriding fact is that the major structural and other features of pollen grains are likely, in all groups, to reflect adaptive function; and here lies the challenge—to identify these features and seek out their roles, or *purposes*, as Darwin himself would not have hesitated to say. The investigation of functional adaptation has no less its fascinations than when Darwin wrote to Thiselton-Dyer in November 1880, "many . . . are very contemptuous about making out the use of organs; but they may sneer the souls out of their bodies, and I for one shall think it the most interesting part of natural history." In this spirit, then, let us consider the adaptive characteristics of the pollen wall.

THE WATER RELATIONS OF THE POLLEN GRAIN

The importance of pores and slits in the wall allowing pollen grains to change in volume was noted as long ago as 1834 by von Mohl, and several other authors of the 19th Century commented on aspects of this function, including Kerner (Kerner & Oliver, 1904) who graphically described the events accompanying pollen hydration and the part played by pores and furrows. Wodehouse (1935) discussed not only the geometry of apertures but also their functions, introducing the term *harmomegathy* to describe the structural adaptations concerned with accomodating to volume change. More recently harmomegathy has been discussed by Payne (1972), who provided an informative table describing apertural mechanisms in a dozen or so families.

The volume changes undergone by pollen grains are occasioned by the loss or gain of water, and it is in control of this that one of the main physiological functions of apertural mechanisms is to be found (Heslop-Harrison, 1971, 1975b).

We can now see that, although lacking the ultimate sophistication of stomata, they are no less effective for regulating the water relations of a single cell. Their action has to be considered through two phases of the life of the grain, during the period of partial desiccation immediately preceding dispersal from the anther, and during the subsequent period of rehydration on the stigma leading up to germination.

THE PHASE OF DESICCATION

Pollen grains vary considerably in their degree of hydration at the time of dispersal. Leaving aside those families in which pollination is actually through the medium of water, there are others with insect- or bird-borne pollens that are hydrated almost to the extent of a somatic parenchymatous cell—for example, certain species of Zingiberaceae and allied families. In the main, however, the grains when released are partly desiccated. The pollen of rye (*Secale cereale*, Gramineae), freshly shed from the anther, may lose up to 35% initial weight when air dried at 80–90°C. This is a high figure when one considers the proportion of fresh weight accounted for by the wall and stored starch and sugars, but it is nevertheless considerably lower than would be lost by a vacuolated somatic cell subjected to the same conditions. Lily pollen fresh from the anther may lose 20–25%, and the pollen of Compositae even less, as low as 15% in some instances. Pollen grains may therefore be dehydrated to the extent of dry seeds at the time of dispersal.

The abstraction of water from the grains occurs immediately before and during the dehiscence of the anther, and this and the drying out of the anther wall cells including the endothecium accounts for the often dramatic fall in anther fresh weight during the hours preceding anthesis (Linksens, 1967). After anthesis, desiccation usually proceeds further, but at a rate very much determined by pollen type, and particularly wall morphology. Grass pollens commonly lose 20% of their initial weight at normal temperatures and humidities within an hour of release, while pollens like those of many Liliaceae and Compositae, already substantially dehydrated before anthesis, show losses of only 2–5%.

To understand the function of apertures in this phase of the life of the pollen grain, three factors have to be taken into consideration,

(a) the pathways of water loss from the male gametophyte;

(b) the capacity of the exine to accommodate volume changes consequent upon water loss and in doing so to control the flow; and

(c) the part played by surface materials in sealing the grain as the equilibrium state is reached.

Setting aside for the moment inaperturate grains and those where the exine is incomplete or absent, it can be said that, in general, water passing in or out of the vegetative cell will have two alternative pathways, through the apertures, or through the nonapertural exine. Since the pathways are in parallel, the total wall resistance, R_w, will equal $\frac{R_a R_e}{R_e + R_a}$ where R_a is the resistance offered by the apertures and R_e that due to the exine. The relationship is comparable with that between stomatal and cuticular transpiration from the leaf.

For the exine in the nonapertural regions the resistance will depend on the

degree of porosity. Fine-structural investigations have revealed that many aperturate pollens have virtually continuous nexines. Compact sporopollenin is essentially impermeable to water, so that where this is true the underlying intine is sealed from water loss or gain. In other pollens, the nexine is penetrated by fine channels, as for example in the grasses (Rowley, 1960). The function of these channels is unknown in any experimental sense, but physical considerations indicate that while there is a continuous liquid phase between intine, exine, and the aqueous environment of the loculus of the undehisced anther, water would be readily extractable through channels the dimensions of those of the grasses, 15–30 nm in diameter, the resistance being only that offered to flow through fine passages.

As for the apertural sites, they, characteristically, are areas of the wall where the exine is much reduced, or virtually absent. Illustrations of apertural exines have been given, for example, by Roland (1966, 1968). These show a variety of states, with sporopollenin deposited in granules or thin plates, very often with marked discontinuities. The intine is thus much more exposed at the apertural sites, presenting a surface that is therefore much less protected from water loss than elsewhere.

The intine itself has many of the chemical and structural characteristics of the primary wall of a normal somatic cell (Sitte, 1953). Like all walls of a pectocellulosic character, it is likely to be freely permeable to diffusing water, and will not therefore offer a serious resistance to water passing into or out of the vegetative cell. Moreover, apart from slight variation in thickness, usually at the apertural sites, the intine invests the grain more or less uniformly. Accordingly, the resistances in the two parallel pathways, through the apertures and through the exine, can be related to the areas of exposed intine; in general, the ratio $\frac{R_a}{R_e}$ will equal $\frac{A_e}{A_a}$, where A_e is the area of intine in communication with external water through the exine channels, and A_a the area in contact through the apertures. For most pollen types, A_a will considerably exceed A_e, emphasizing the importance of the apertural pathway, which will be the only one when the exine is impermeable.

During the first period of partial dehydration in the anther, the developing pollen lies immersed in the locular fluid. Water will be withdrawn along water potential gradients occasioned by deficits developed elsewhere in the anther, filament or receptacle. In many pollens, of which those of the Liliaceae and Gramineae provide good examples, starch accumulates at this time, and this will presumably steepen the gradient by sequestering osmoticum and raising the water potential.

The rate of water loss will be determined by the resistance offered by the pollen wall, and the control of exposed intine area at the apertures will be a crucial factor. It is in the regulation of this that the apertural mechanisms play their part during the phase of dehydration. The devices are diverse, but all are related to the change in volume of the grain during desiccation (Heslop-Harrison, 1971). A classification of some of the more important mechanisms is given in Table 1. All serve to reduce the area of the intine in contact with the locular fluid during dehydration and contraction; they act therefore to increase R_a and so

TABLE 1. Some examples of apertural mechanisms.

Exine Type	Sealing Devices
Colpate; sulcate	(a) Infolding, with buckling, folding or interleaving shutters; lipid seal (e.g., *Lilium*)
Porate	
Single pore	Operculate; no lipid seal (Gramineae)
Multiple pores	(a) Operculate; with or without lipid seal (e.g., Caryophyllaceae) (b) Occlusion by sporopollenin granules; lipid seal (e.g., Malvaceae)
Colporate; triaperturate	Infolding, with simple folding or buckling shutters or interleaving sporopollenin plates; with or without lipid seal (commonest dicotyledonous types)
Inaperturate	Granulate exine; lipid seal (e.g., many Iridaceae)

progressively to restrict water flow. At some point, however, the continuity of the liquid phase in the loculus will be broken, usually shortly before, or at the time of, the dehiscence of the anther. The pollen grains then find themselves in an aerial environment; and certainly by the time of release this establishes a new situation where the forces withdrawing water are not those imposed by water potential gradients in the stamen but by those of evaporation, many times greater in magnitude. The capillary forces holding water in channels as narrow as those found in the exine when this is porous are such as to ensure that they will not empty before the water content of the adjacent intine has reached an extremely low level. However, the menisci formed between the sporopollenin plates and granules at apertural sites may well have larger effective dimensions, and under the evaporative forces likely to be encountered on a normal day the air-water interface might be expected to withdraw to the nearest intine surface, where evaporation would be from the interfibrillar spaces. This would no doubt reduce the hydraulic conductivity still further, but loss would still be rapid and could lead very quickly to a lethal degree of desiccation.

This is resisted by still another adaptation, for it is in the control of water loss during the period immediately following the exposure of the pollen that lipid surface materials make one of their most important contributions. These lipidic materials are synthesized in the tapetum and transferred to the exine during the final period of pollen maturation. They show a marked selectivity in the walls to which they adhere (Heslop-Harrison, 1968). In *Lilium*, for example, they disperse preferentially over the spaces between the muri of the reticulate exine, and particularly over the colpus. In the latter site a globule is usually entrapped as the margins draw together during the final dehydration of the grain. This selective affinity may indicate the presence of a lipoprotein which forms an interfacial layer over the residual polysaccharide of the primexine matrix in the meshes of the exine reticulum, and over the intine itself where this is exposed.

The consequence of the coating of the exine and apertural sites with lipid is dramatic, for such a coating both seals the micropores of the exine where these are present and contributes greatly to increasing the hydraulic resistance of the

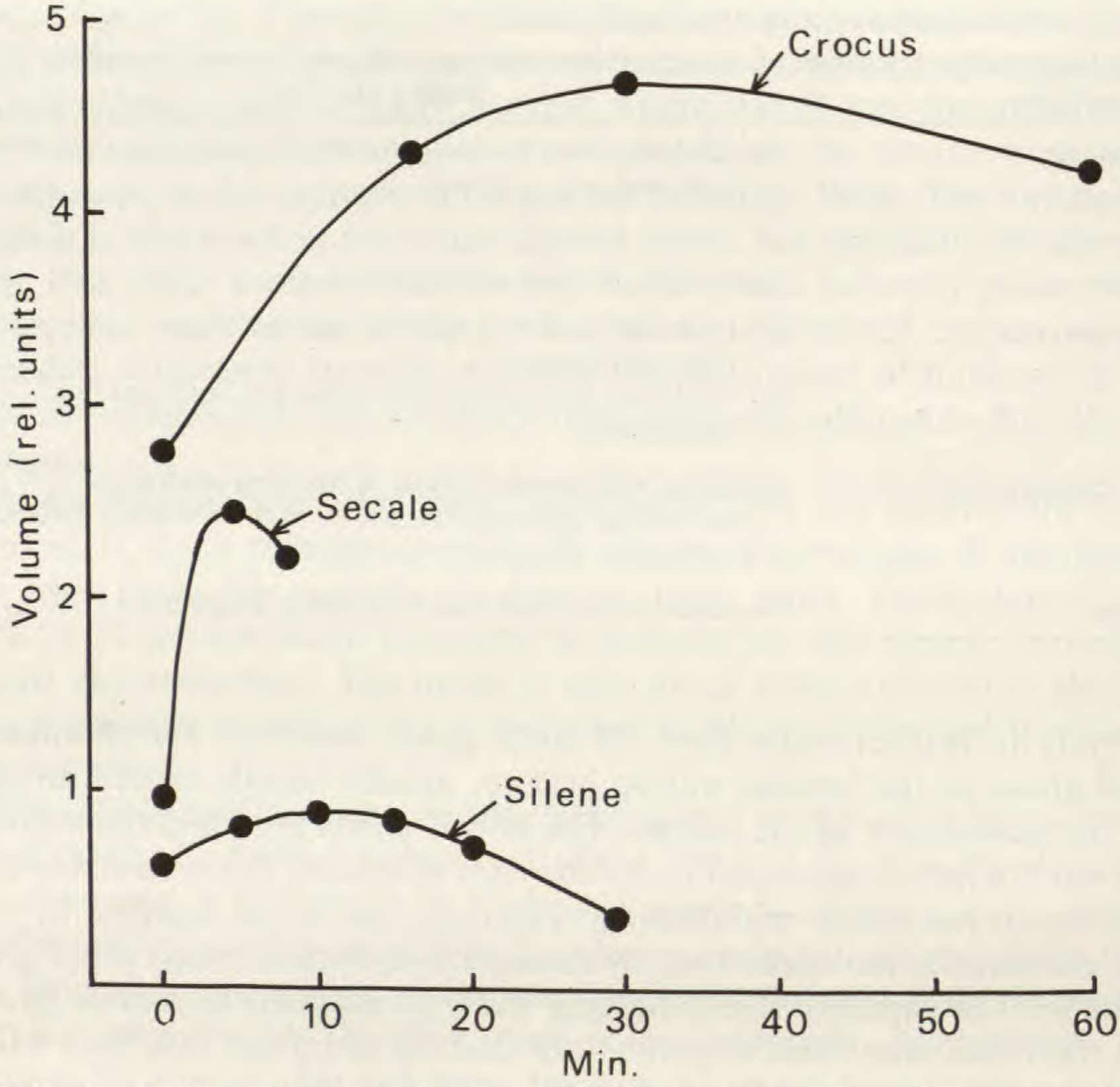


FIGURE 1. Volume changes during hydration in the pollen of *Crocus chrysanthus* (Iridaceae), *Secale cereale* (Gramineae), and *Silene vulgaris* (Caryophyllaceae). In all instances the volume of the grain itself is recorded; the tubes emerged at the point of maximum volume in each.

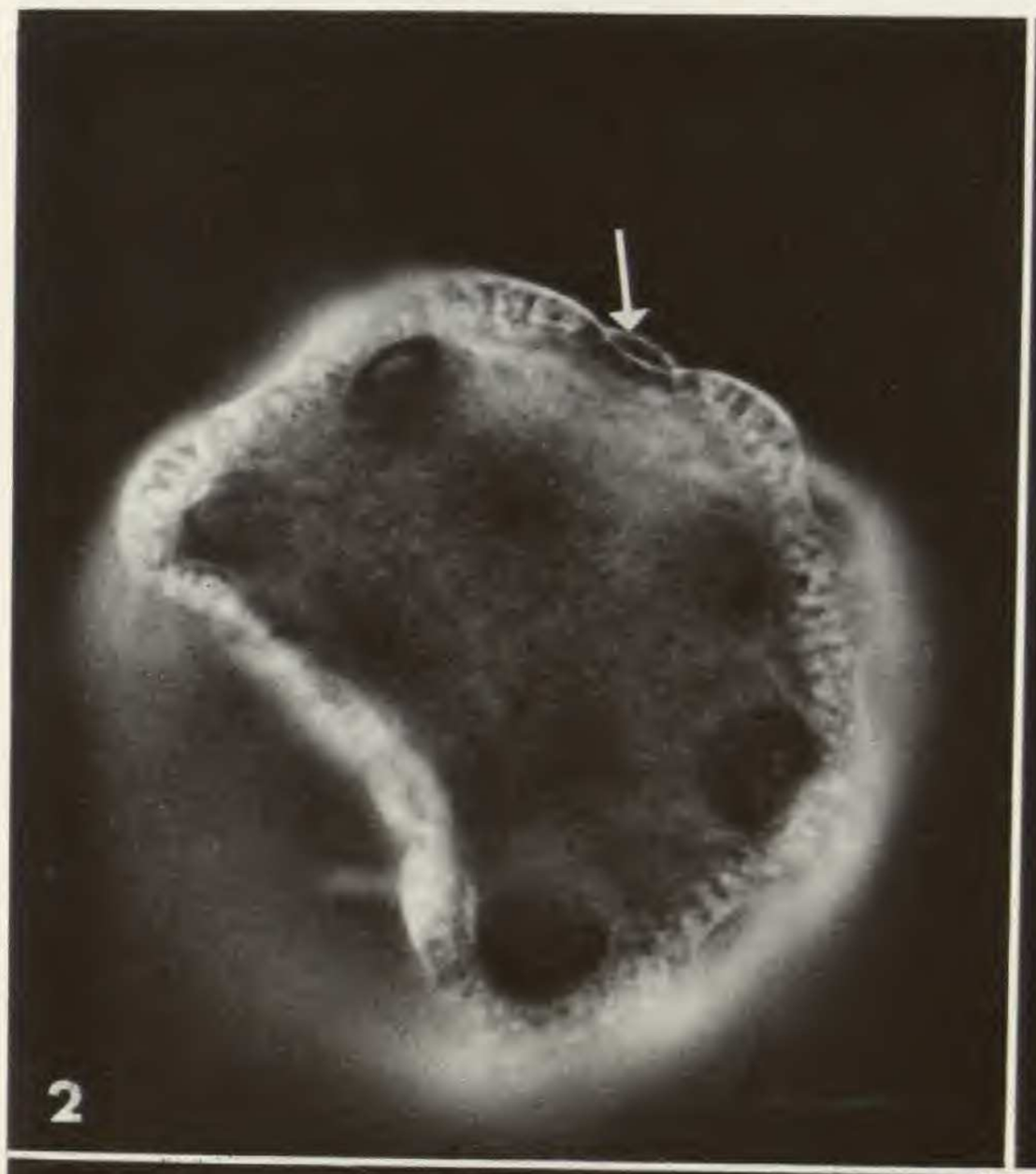
aperture sites. It is significant, therefore, that lipidic surface materials are present in most pollens. The exceptions, where the surface carries little surface lipid, are cases where the pollen is dispersed through water or saturated atmospheres—or where the pollen does indeed dehydrate quickly after release and has a very short functional life, as in the grasses and certain other wind pollinated groups.

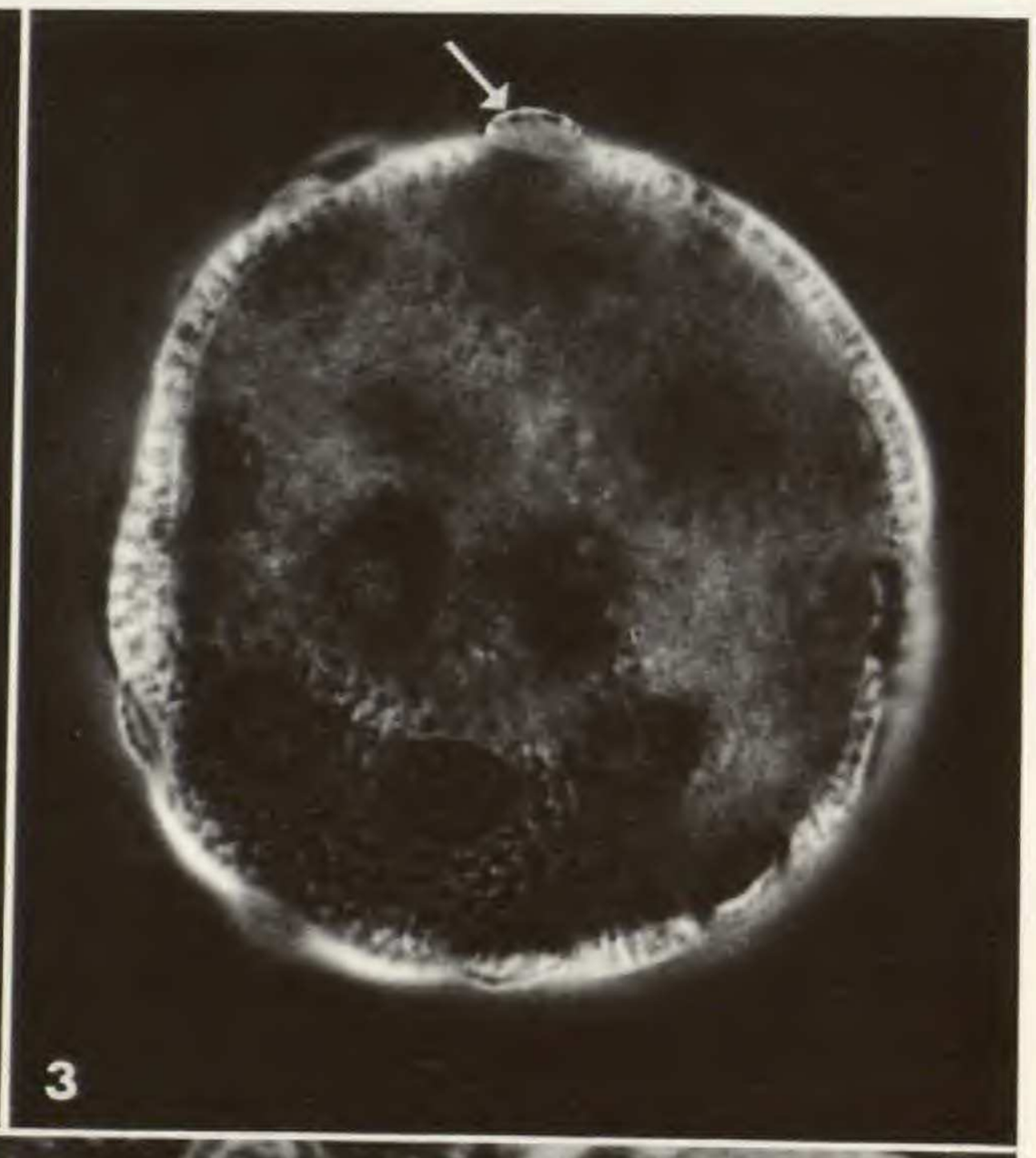
THE PHASE OF REHYDRATION

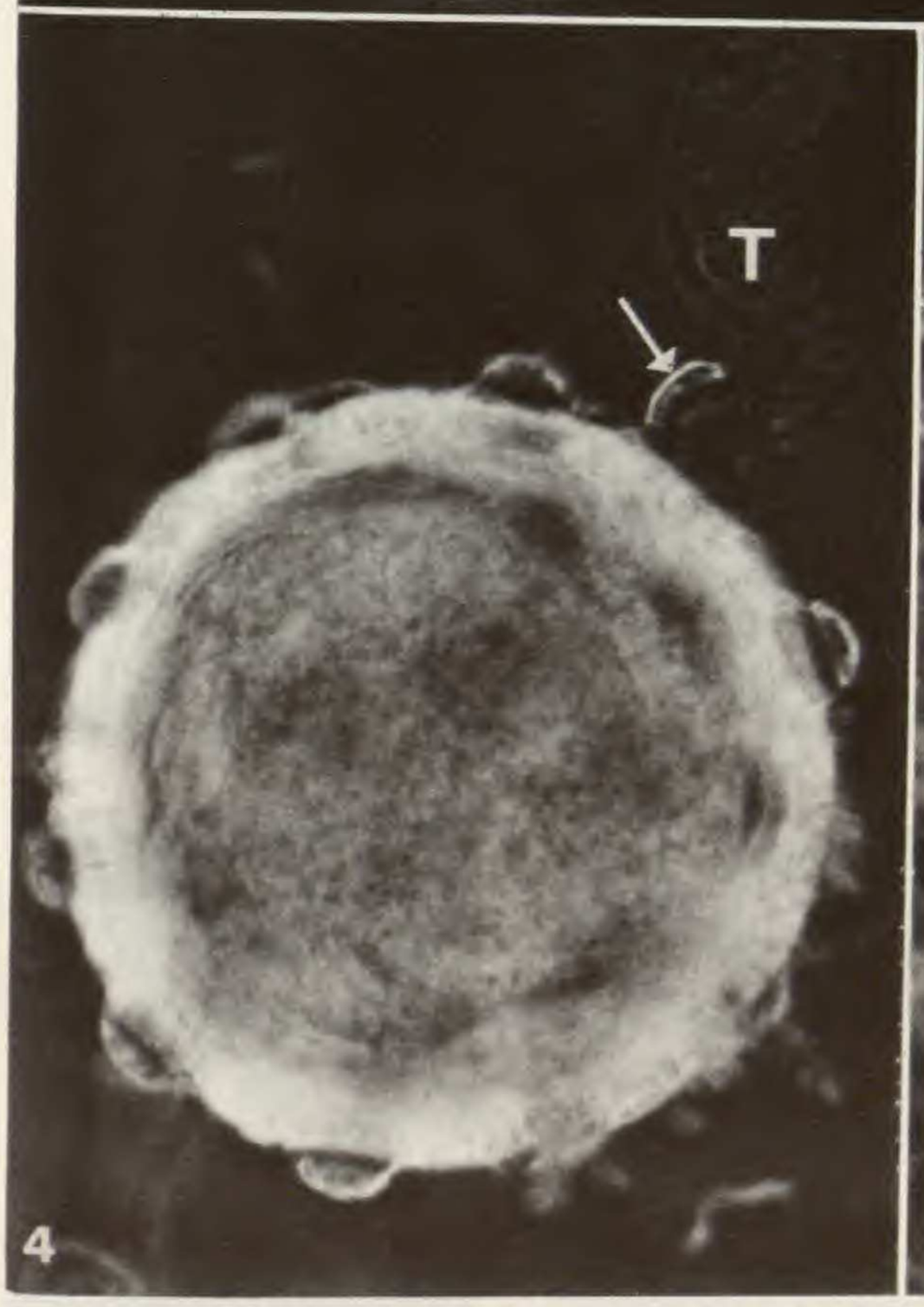
The fate of a successful pollen grain is to alight on a suitable receptive stigma. The receptive surfaces of stigmas are found in many structural and physiological states (Y. Heslop-Harrison & Shivanna, 1977), but all must provide the conditions for rehydration of compatible pollen as an essential preliminary to germination.

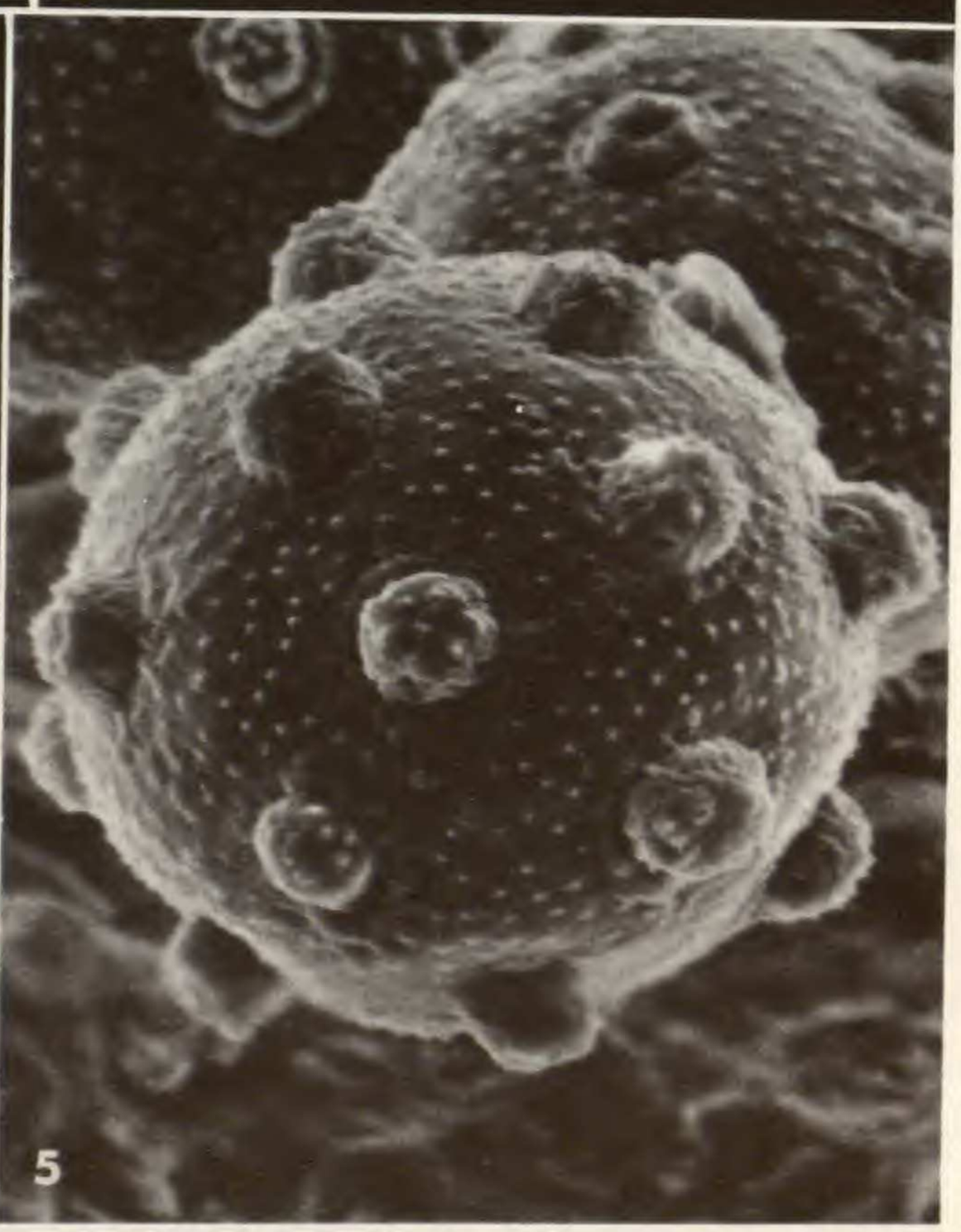
Rehydration reverses the changes that occur during the final period in the anther, but we can say a good deal more about the details because the events can be observed much more readily.

The time course of rehydration preparatory to germination is shown for three species with different intine types in Fig. 1. These measurements were made directly on the stigma using time lapse photography. All of the species have "dry" stigmas (Y. Heslop-Harrison & Shivanna, 1977), so that the grains abstract

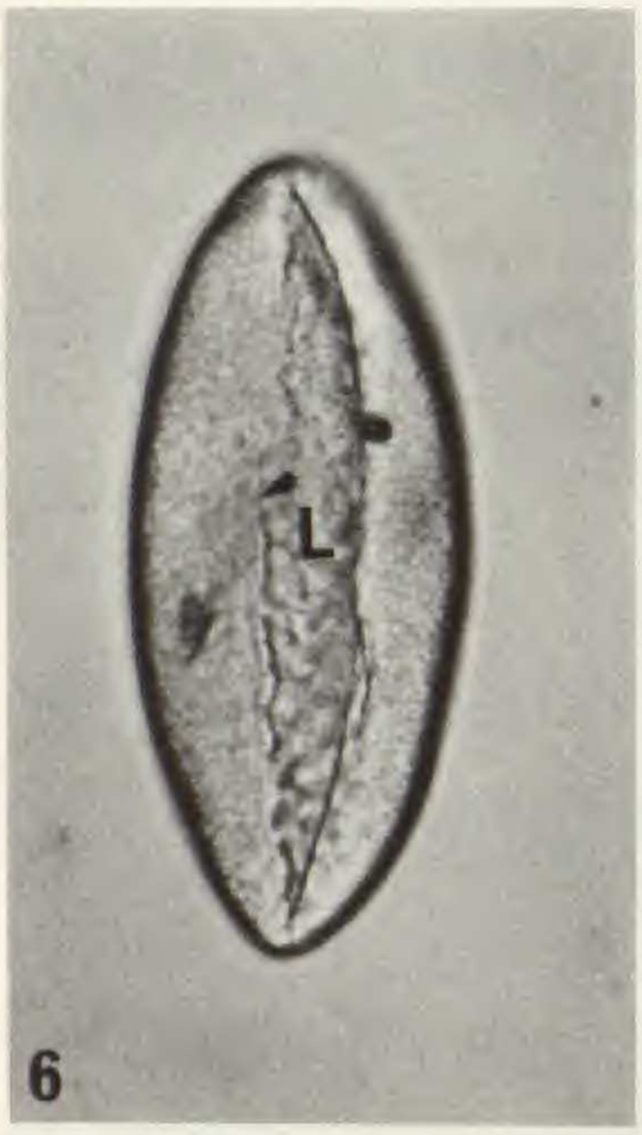


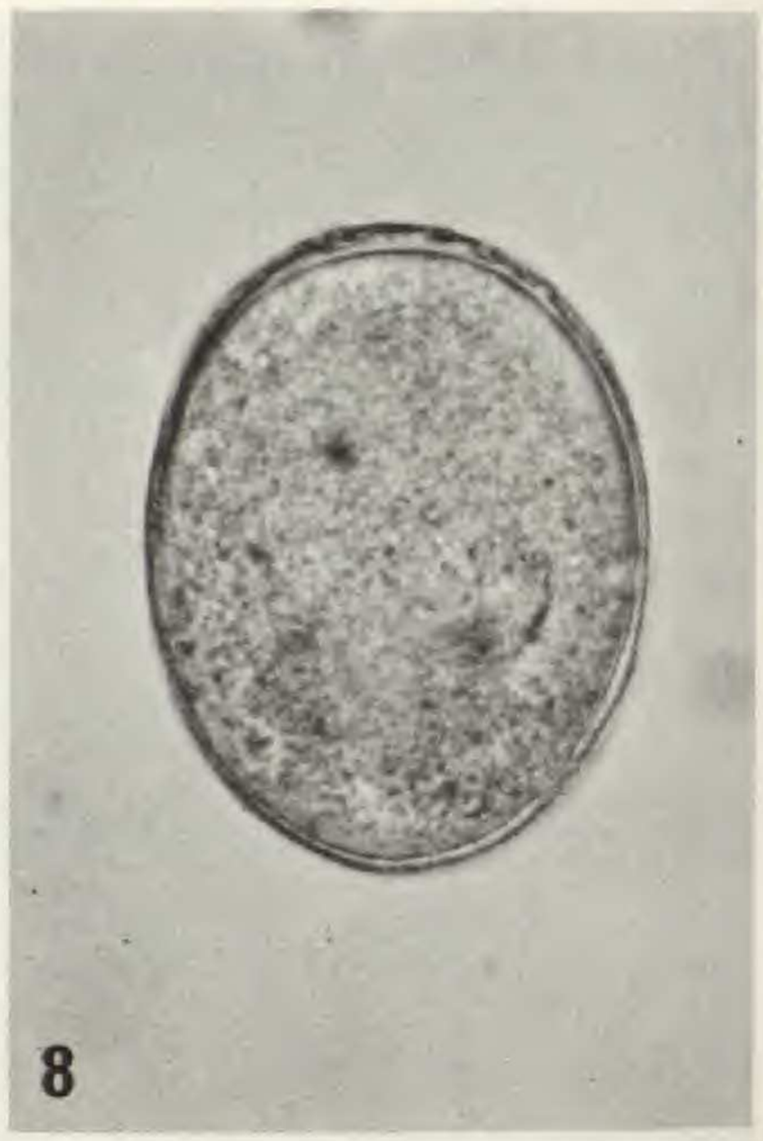


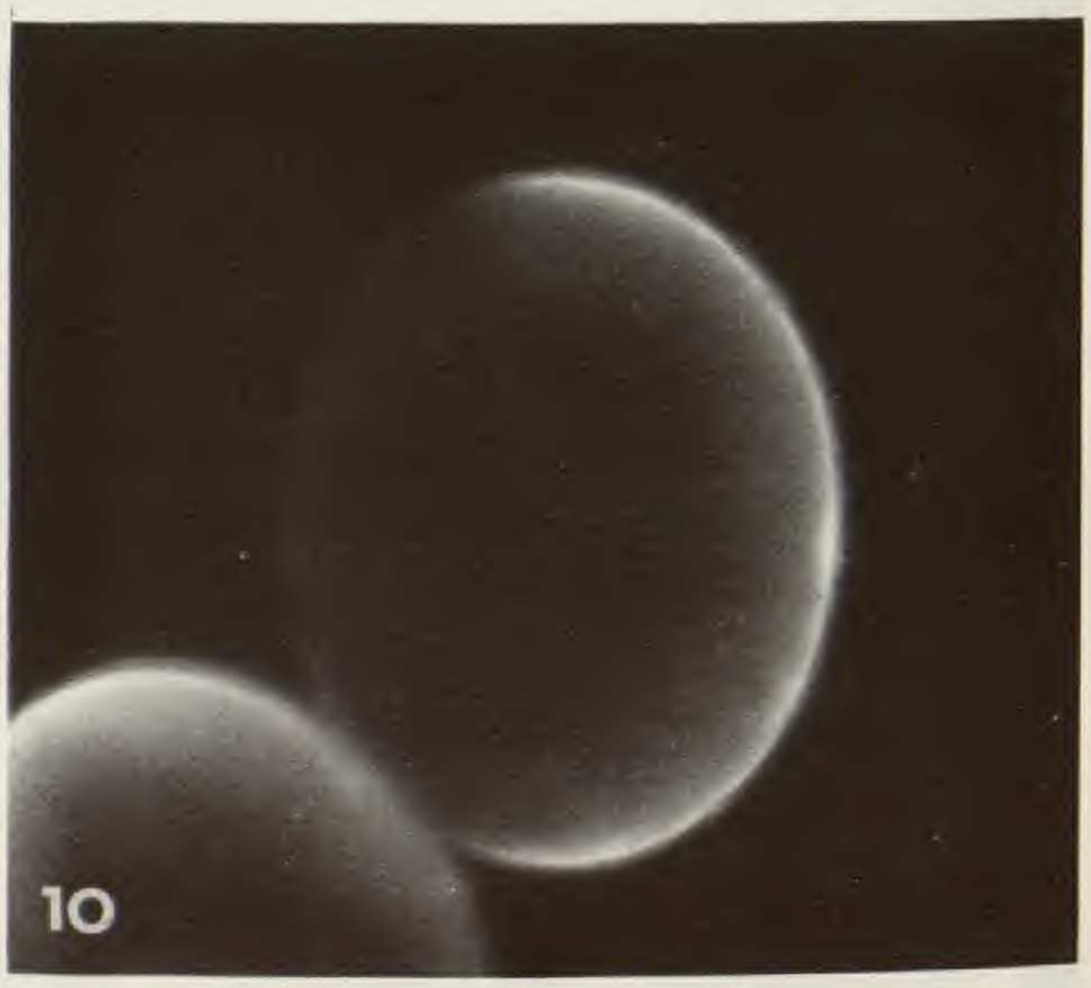


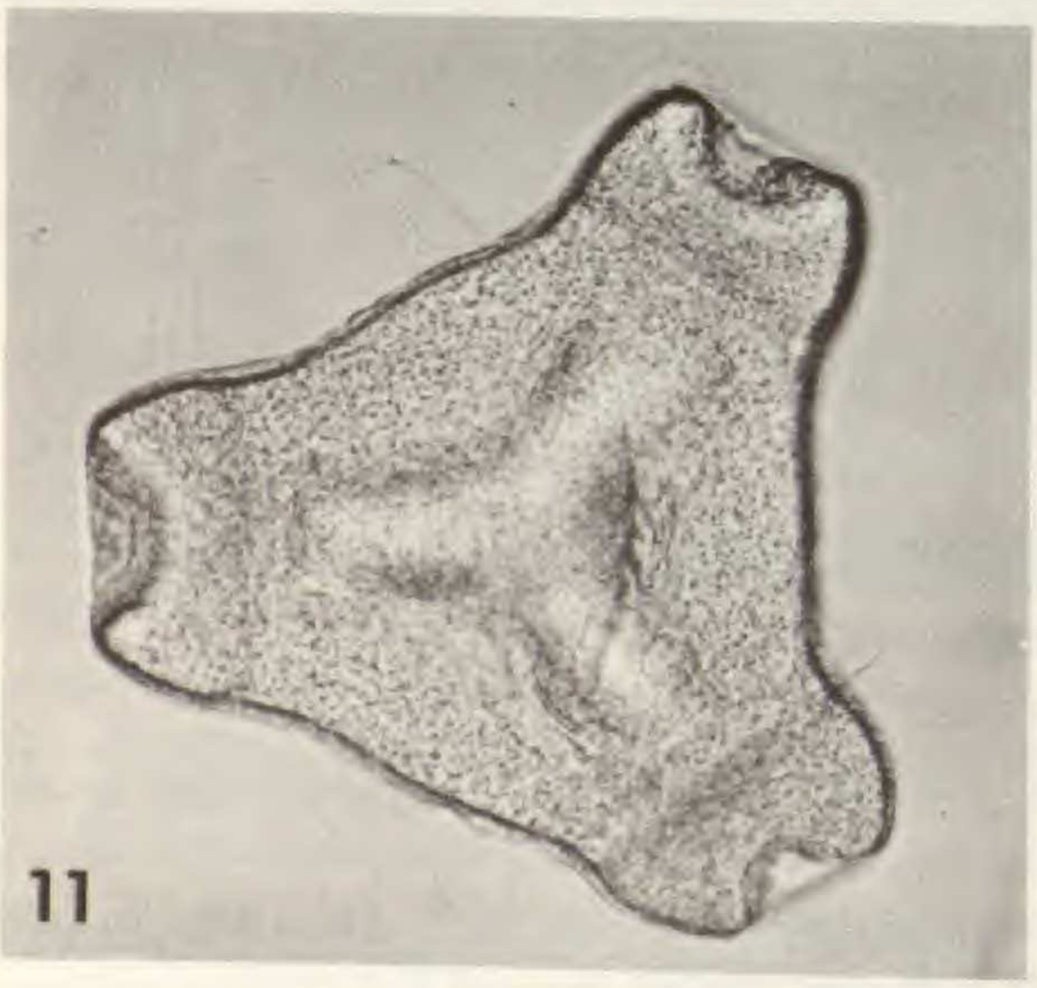


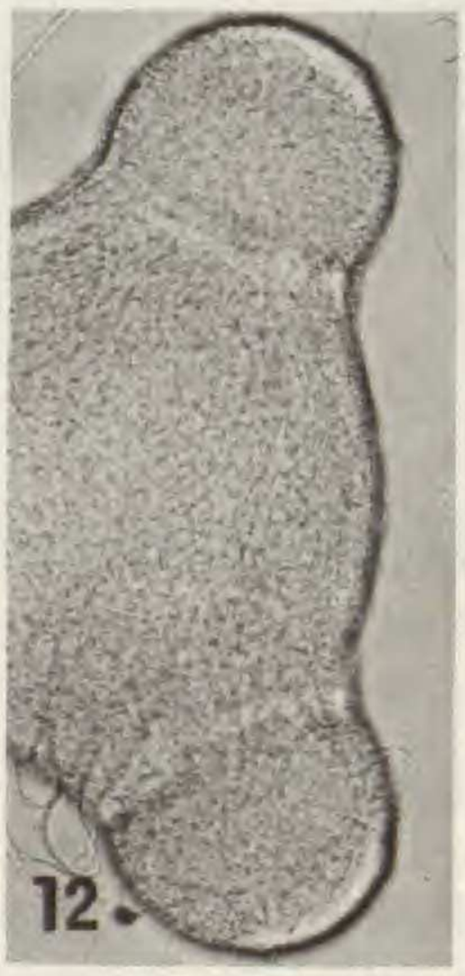
FIGURES 2–5.—2. Pollen grain of *Agrostemma githago* (Caryophyllaceae) suspended in a polyethylene glycol solution of an osmotic pressure of 5.7–6.0 MPa. The arrow shows an operculum, pressed into the surrounding sporopollenin annulus and so offering an effective seal; ×650.—3. As Fig. 2, grain suspended in a medium that allows some expansion of the grain. The arrow points to an operculum which is now rising slightly under the pressure of the underlying intine; ×650.—4. Grain in a germination medium that allows normal hydration. An operculum has lifted (arrow), and the pollen tube (T) has emerged; ×650.—5. Scanning electron micrograph of a pollen grain of *Agrostemma githago* in a state of full imbibition, showing the elevated opercula and exposed exines at each apertural site; ×700.

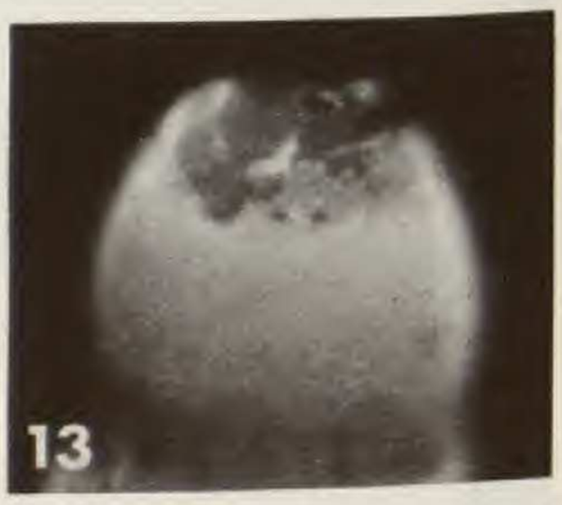












FIGURES 6–14.—6. Pollen grain of *Iris* (Iridaceae) suspended in a polyethylene glycol solution of an osmotic pressure of ca. 5.5 MPa. The grain has scarcely enlarged, and the lipid seal still lies over the colpus (L); ×425.—7. As Fig. 6, grain in a medium that allows some hydration. The lipid over the colpus is beginning to disperse; ×425.—8. Grain in a germination medium that allows normal hydration; ×425.—9. As Fig. 8, fluorescence micrograph after auramine O staining to reveal the

all of the water from the contiguous stigma papilla. The hydration and germination rates differ considerably among the three. Maximum grain volume was attained in 2–3 min in *Secale cereale*, but not until 35 min in *Crocus chrysanthus*. In each species, the attainment of full volume was followed by some shrinkage as the pollen tube emerged. In the case of *Silene vulgaris*, the grain was oriented so as to appose a germination aperture directly to the stigma surface. Grains oriented differently do not hydrate so rapidly (Heslop-Harrison et al., 1975). Orientation on the stigma is less important for the pollen grains of grasses; although there is only one germination aperture, both layers of the exine are penetrated by channels through which hydration takes place.

The ingress of water and the consequent dilation of the grain results in changes in the shape and area of the apertures. The events accompanying the dehydration before and during dispersal are, in fact, repeated in reverse. They can be followed on the actual stigma surface, or rather more conveniently on "artificial" stigmas, for example on membranes floating on germination medium, or in some instances actually in the liquid medium.

We may take as an example the pollen of *Agrostemma githago* (Caryophyllaceae). The grain freshly shed from the anther undergoes no volume changes in a solution of polyethylene glycol of a molecular weight of 400 daltons adjusted to have an osmotic pressure of 5.7–6.0 MPa. At this stage, the opercula are fitted closely into the apertures, which are somewhat depressed marginally so that the grain is at minimum volume (Fig. 2). In solutions of lower tonicity, the grain expands, and the opercula are lifted, so exposing more of the intine (Fig. 3); finally, after full hydration the intine is pushed out at each apertural site (Fig. 5), and this is the prelude to germination (Fig. 4). In *Agrostemma*, only small amounts of lipidic material are transferred from the tapetum to the exine during the maturation of the pollen, and these are dispersed early during hydration, rapidly exposing the apertural intine.

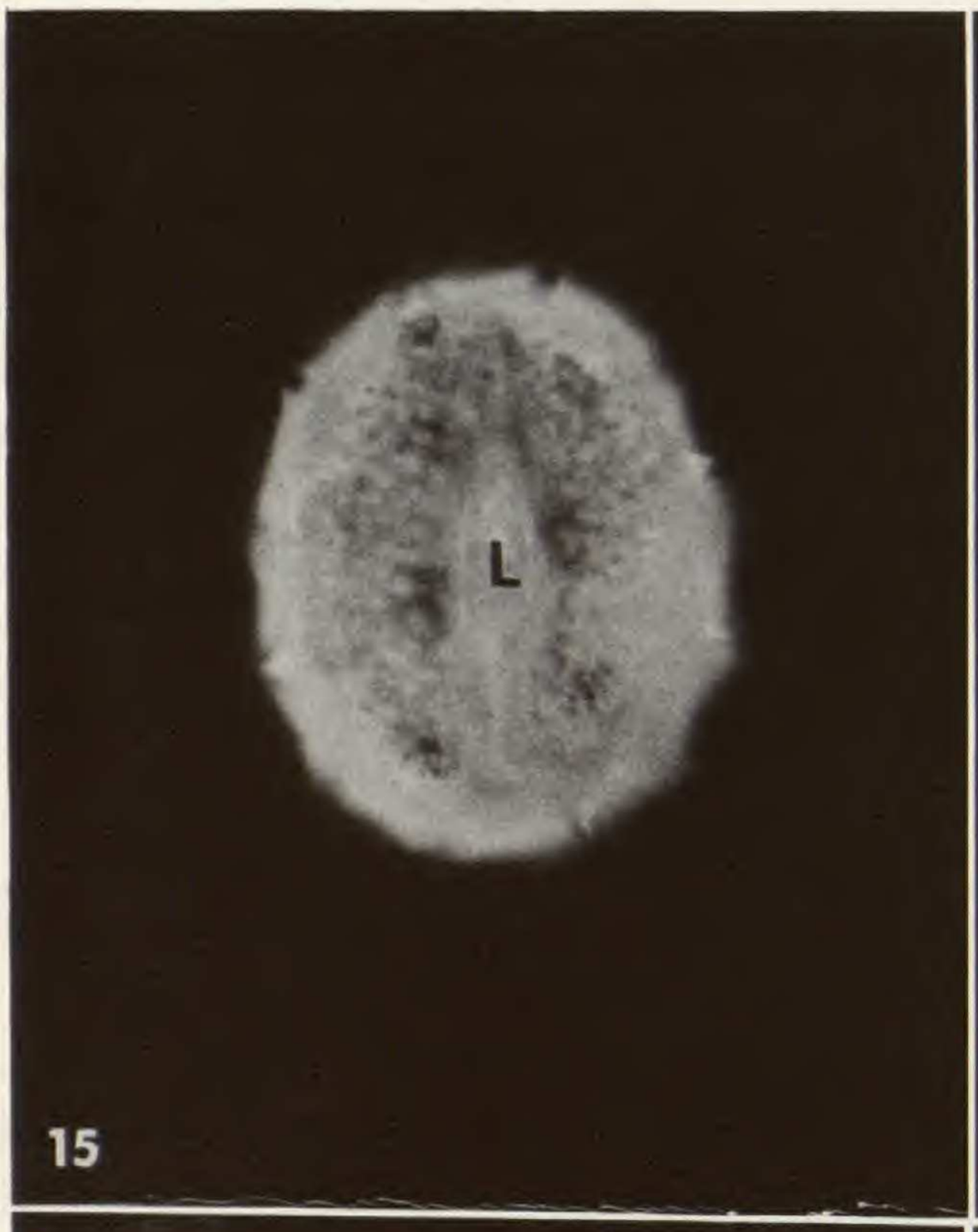
Obviously, the implication of these changes is that the more the grain hydrates, the lesser the hydraulic resistance to further inflow of water. Uptake is therefore governed by a neat feedback mechanism. The sequences of Figs. 6–10, 11–14 and 15–17 illustrate how the same principles apply to colpate and tricolporate grains. In two of these examples, the colpi were initially sealed by the tapetally derived lipids. These are dispersed in the bathing medium; the colpi then progressively gape, exposing more and more of the intine until finally the exposed surface accounts for some 30% of the total area of the grain in the colpate grain of the iris and 40% of the tricolporate grain of *Tagetes*.

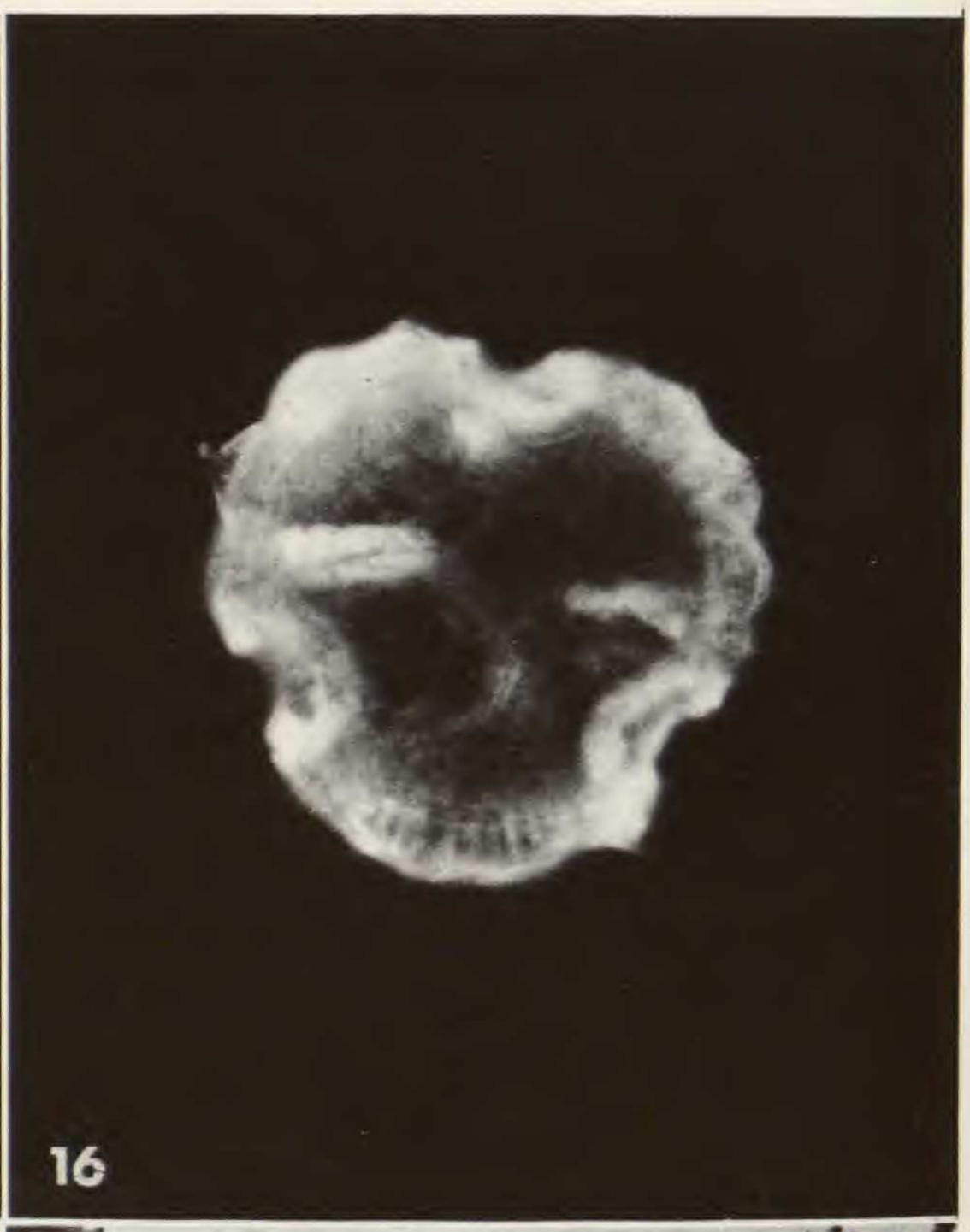
The pathways of water uptake during initial hydration can be identified by

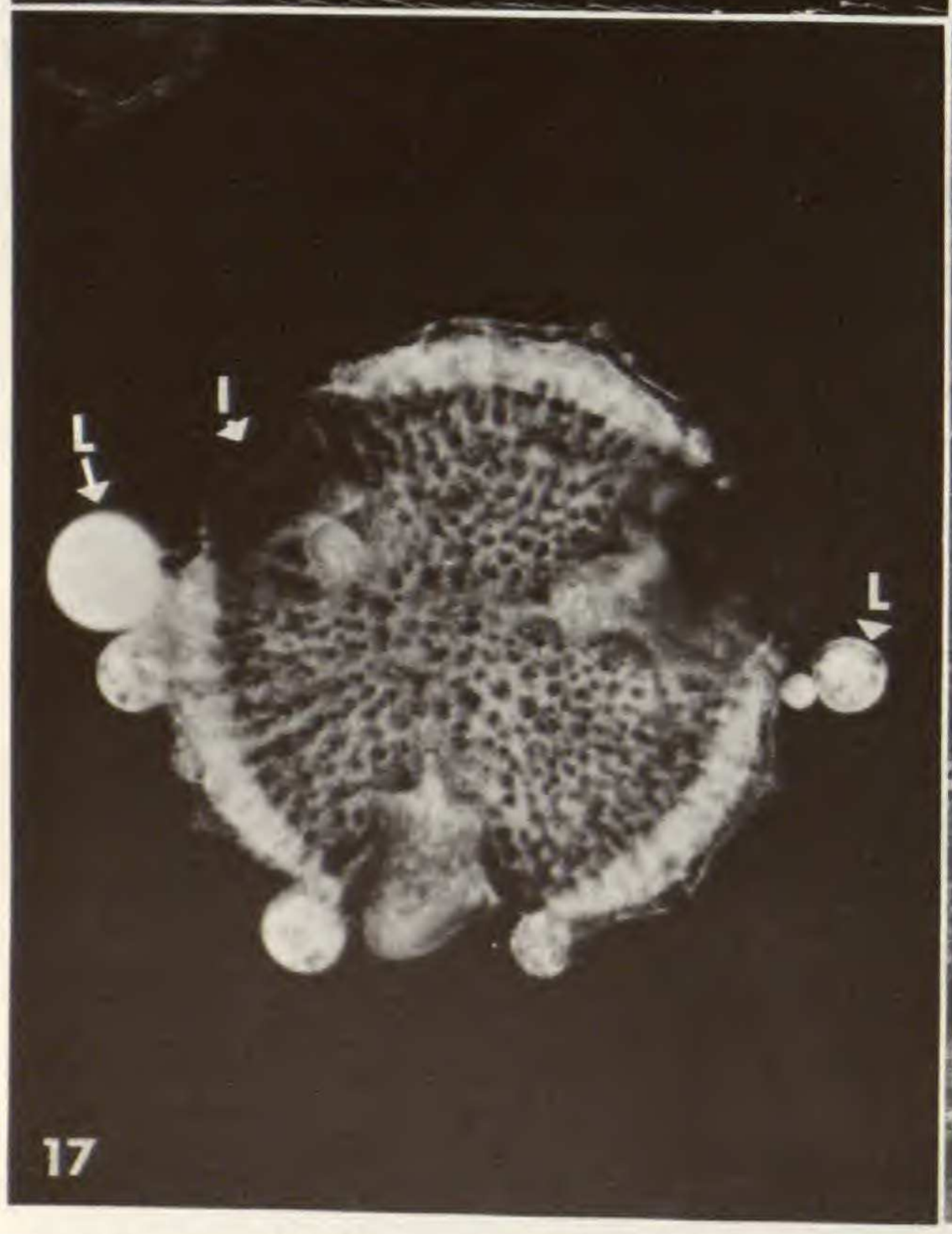
---

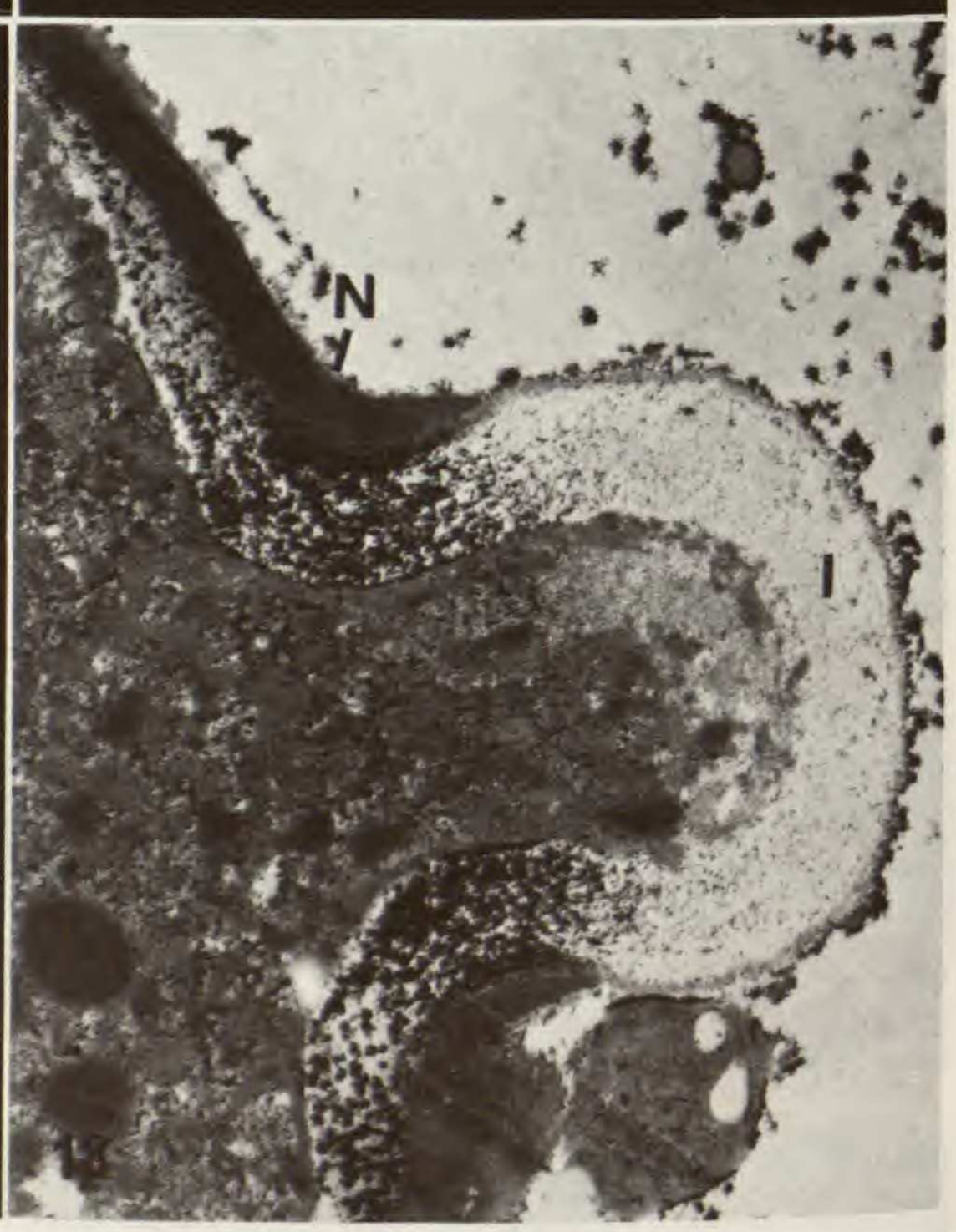
←

exine; ×400.—10. As Fig. 8, fluorescence micrograph after calcofluor white staining to reveal the apertural intine, now fully exposed; ×400.—11. Pollen grain of *Oenothera organensis* suspended in a polyethylene glycol solution of osmotic pressure ca. 6.0 MPa. The exine is infolded at each apertural site. The seal in this case is given by numerous interlocking sporopollenin lamellae in the throat of each aperture; little surface lipid is present; ×300.—12. As Fig. 11, grain suspended in a germination medium. The intine at the apertural sites is now everted, and the sporopollenin plates are torn apart; ×300.—13. As Fig. 12, auramine O staining to show the torn exine; ×350.—14. As Fig. 11, calcofluor white staining to show emergent intine; ×350.









FIGURES 15–18.—15. Pollen grain of *Tagetes patula* (Compositae), suspended in a solution of polyethylene glycol with an osmotic pressure of ca. 6.0 MPa. The grain has undergone no expansion, and the three colpi are still closed and sealed by lipid (L); ×800.—16. As Fig. 15, polar view showing the three colpi; ×800.—17. Pollen grain of *Tagetes patula* in a medium allowing normal hydration. The aperture sites are now gaping, and the underlying intine (I) is revealed. The lipids (L) are dispersing into the medium; ×800.—18. Electron micrograph of an aperture of a pollen grain of *Cosmos bipinnatus* (Compositae), after hydration in a medium containing the electron-opaque tracer, colloidal lanthanum nitrate. The intine (I) has emerged from the aperture as it would in a normal germination. The tracer has, however, entered through the aperture in the early period of hydration, and is present underneath the adjoining nexine (N). The nexine is itself impermeable to the tracer. The presence of the tracer inside the protoplast of the vegetative cell suggests that the plasmalemma does not form an effective barrier during the early stages of hydration; ×4,500.

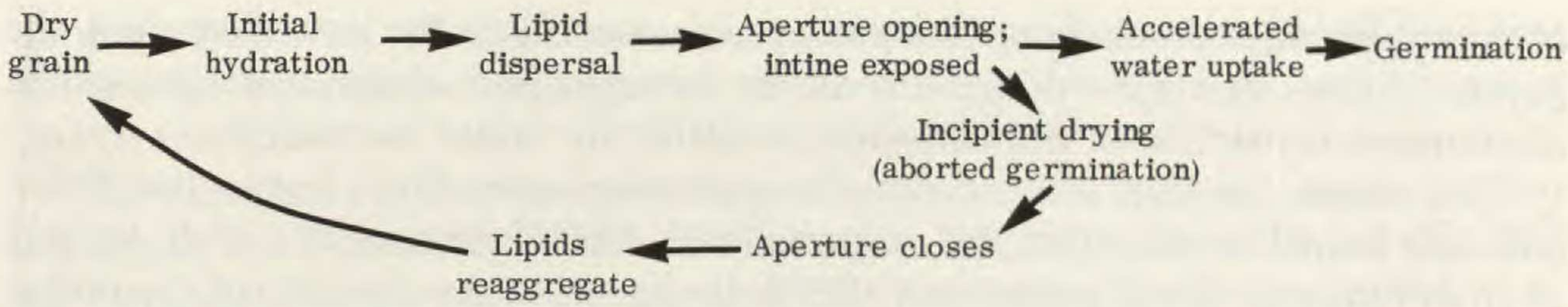


FIGURE 19. Normal sequence of hydration in pollen grains up to the point of germination.

following the movement of suitable electron-opaque tracers. Figure 18 shows the distribution of colloidal lanthanum nitrate supplied to the pollen of *Cosmos bipinnatus* at the time of first exposure to the hydrating medium. The tracer moves into the sexine and cavea, but does not penetrate the nexine, which is without channels in this species. The principal passage into the grain, however, is through the aperture sites. The tracer passes freely into the intine, and is dispersed tangentially through it under the nexine neighboring the apertures, as well as moving into the vicinity of the plasmalemma.

An important feature of most of the more elaborate apertural regulating mechanisms, and one of undoubted adaptive value, is that their function is reversible almost up to the point where the grain actually germinates. This can be demonstrated very readily using artificial stigmas consisting of thin gel layers. On these, the pollen grains can be taken through successive cycles of low and high atmospheric humidity. Viability is progressively lost, but many species survive two or three such cycles.

The normal sequence of hydration up to the point of germination is summarized in Fig. 19, which also shows how, up to a certain point in the time before the actual emergence of the tube, the sequence may be reversed. Figure 19 takes no account of the exudation phase which, as we shall shortly see, may be interpolated early in the period of hydration.

REGULATORY FUNCTIONS OF THE STIGMA

The source of the water entering the pollen is of course the stigma. As we have already noted, the receptive surfaces of angiosperm stigmas are highly diverse, but a broad division may be made into those which bear a free fluid surface at maturity ("wet" stigmas) and those without a free-flowing secretion pool ("dry" stigmas). While in the receptive state stigmas are more or less freely exposed to the atmosphere, so all must be subject to considerable evaporation. As yet we are without data for the evaporative loss from wet stigmas, but this might be expected to vary considerably according to the effectiveness of lipid coatings (Konar & Linskens, 1966) and to the concentration of osmoticum in the secretion.

Dry stigmas have the capacity for regulating water loss through the surface of the papillae by varying the hydraulic resistance of the cuticle (Heslop-Harrison, et al., 1975; Heslop-Harrison, Heslop-Harrison & Barber, 1975). The stigma papilla is a secretory epidermal cell with a pectocellulosic wall limited by a cuticle, but the cuticle, being made up of radially oriented cuticularized rods or columns, is always discontinuous. In the turgid papilla, the rods are drawn apart on the surface

of the expanded wall, allowing free passage of water. With the loss of turgidity the papilla shrinks and the cutinized rods are brought into closer contact, with a consequent restriction of the pathways available for water movement.

The stigma has thus the capacity for regulating water flow, just as the pollen has, and it will be seen that the system allows for adjustment to a wide variety of circumstances (Heslop-Harrison, 1979). It conserves the capacity of the pollen grain to resist excessive desiccation right up to the moment when circumstances are propitious for germination and the entry of the tube. For example, a plant in a state of water stress may receive pollen, but will be unable to provide the conditions for hydration until an appropriate water balance has been achieved in the stigma. At this point the pollen will be permitted to begin hydration, and at an increasing rate, as long as the water flux from the stigma continues. Should the flow be inadequate to support full hydration, the sequence will reverse, the grain will begin to dry out and the apertures will close once more. Such an episode need not necessarily affect the possibility of future success when the water balance of the stigma is restored.

MEMBRANE PERMEABILITY, DESICCATION AND HYDRATION

With a few rare exceptions, viable angiosperm pollen grains lack vacuoles at the time of dispersal. This implies that the water potential of the grain is likely at first to be established by the matric potential attributable to the colloids of the protoplast and wall and the solutes present in the cytoplasm of the vegetative cell. In the desiccated grain, a cell "membrane" as such is unlikely to exist, the membrane lipids being distributed in micelles in the condition of minimal water content—comparable, that is, with the membranes of desiccated seeds (Heslop-Harrison, 1979). In the system overall, the only effective semipermeable membranes will thus be those of the stigma papilla.

After hydration has begun, however, the membranes are quickly reorganized in the pollen grain. In rye, this can take place in two to three minutes, at which time the first movements of the protoplasm can be seen in the grain. Vacuolation occurs in under 5 min in rye, and by this time germination will normally have begun, and normal tonoplast, plasmalemma, and endoplasmic membranes can be seen with the electron microscope. The periods are undoubtedly longer for other species (Fig. 1), but the sequence of events is always the same in those pollens that are dispersed in a partly dehydrated state. The later growth of the tube and the transfer of cytoplasm and reserves from the grain into it is accompanied by the enlargement of the vacuolar system, and this must also be supported by transfer of water from the stigma until such a time as the pollen tube has entered the transmitting tract.

The water potential of the grain when first in contact with the stigma will be influenced not only by osmotic forces but by pressures attributable to the wall. In the dehydrated grain at minimum volume the wall will apply a tension to the protoplast of the vegetative cell. During the rehydration of the grain, a point will be reached when the wall exerts no pressure, and with further inflow of water the pressure will become positive. While the membranes of the vegetative cell remain ineffective as osmotic barriers, this pressure will be exerted only on the protoplast, in effect balancing matric potential. Further passage of water will

continue, however, while the water potential of the grain remains lower than that of the stigma papilla in consequence of the solutes of the pollen, and this must lead to exudation.

The transition to this phase is seen spectacularly in the grasses, an observation first made by Watanabe (1955). Exudation is first seen from the germination aperture, and then through the micropores of the exine. In rye the outflow may account for as much as 15% of the volume of the grain.

When the membranes of the pollen grain have become reorganized, the vegetative cell begins to act like any somatic cell of the plant, and the familiar laws governing plant-cell water relations begin to apply. Containment of the solutes in a semipermeable membrane restricts their movement into and through the wall, and further endosmosis will be exclusively *into* the grain. Exudation will then cease, and further expansion will begin; but in the normal course of events this will mark the moment of germination, and enlargement in volume will be in consequence of the growth of the tube.

Apertural Mechanisms and the Function of the Wall as a Repository

In many angiosperm families the pollen wall conveys enzymes and other proteins (for reviews of this function, see Heslop-Harrison, 1975a, 1975b). The protein load of the *exine,* like its surface materials, is derived from the tapetum of the anther, and that held in the *intine* is inserted during the thickening of this layer of the wall in the spore and young pollen grain, the source being the male gametophyte itself. In aperturate grains, the exine materials are held in the cavities and sculpturings of the sexine in the nonapertural areas, while the intine protein load is concentrated in the region of the apertures (Heslop-Harrison, et al., 1973).

In families with aperturate grains and exine storage—such, for example, as the Cruciferae and Compositae—proteins from the exine domain are released very soon after the beginning of hydration, passing out onto the stigma surface and associating there with materials from the stigma cells, while the intine proteins are released only after the grain has become partly hydrated (see, for example, Howlett, et al., 1973).

Consideration of the sequence of events accompanying the initial hydration shows why this should be so. At first, the net flux of water will be *into* the grain at the apertural sites as the intine is progressively exposed. As full turgidity is approached, the tide will be turned; the phase of exudation follows, and for a while the net flux is outwards from the grain. At this time mobile fractions held in the apertural intine will be flushed out onto the stigma surface.

Apertural mechanisms thus play a part in regulating the outflow of intine-held proteins. There seems to be something of a compromise, here, however, for such devices could act to prevent premature release only in the first period of hydration; once the phase of exudation is reached one might expect mobile constituents of the intine to be lost irrevocably. The functions of the enzymic load of the apertural intine are still to be fully explained, but two roles are attributable to them, (a) in the softening of the intine at the germination site, as essential prelude to the emergence of the tube tip (Stanley & Linskens, 1974; Konar & Stanley,

1969), and (b) in the early interactions with the stigma, most probably during the penetration of the cuticle and perhaps in the early nutrition of the pollen tube (Heslop-Harrison, 1971). A further possibility is that the intines of certain species carry proteins concerned with the recognition reaction of gametophytic incompatibility systems (Heslop-Harrison, 1978), but in no case has this been shown experimentally as yet.

In certain advanced pollen types, the germination apertures are equipped with devices facilitating the penetration of the exine and the emergence of the tube tip. In the grasses and various other families with operculate apertures, the first stage of germination involves the Zwischenkörper, an outer layer of the intine, composed of pectic substances, underlying the operculum at the aperture. With the beginning of hydration, the Zwischenkörper swells to form a gel, and it is this gel that first lifts the operculum. The emerging tube tip pushes through the gel, which is then dispersed. Germination is prevented if the calcium ion concentration of the medium is raised beyond the optimum, apparently through the rigidification of the pectins of the Zwischenkörper. The same result is obtained if an appreciable proportion of the intine protein is leached out in a medium of suitable tonicity before the conditions for germination are offered. In each case the grain is "switched on" metabolically speaking, but fails to produce a tube. That the treatments are in no sense lethal to the protoplast is shown by the persistence of cytoplasmic streaming and the progressive digestion of storage starch.

Presumably the intine enzymes are involved both in the dispersal of the Zwischenkörper and in the early relaxation of the underlying cellulosic part of the wall essential for the formation of the tube tip. Their loss therefore blocks germination. From these experiments with grass pollens one may deduce the reason for the fact that, in general, pollens lose viability on leaching even when they survive the treatment in a structurally intact state.

WHY SEVERAL APERTURES?

Monocotyledons characteristically have a single germination site, and most dicotyledons have three; but trends towards higher numbers have seemingly arisen several times in the evolution of flowering plants. Presumably some functional significance attaches to this.

Certain correlations can be suggested. Although adequate statistical data are not available, I suspect that it would turn out that the ratio of apertural area, measured as mean area of aperture × mean number per grain, to total wall area would vary less among and within the various groups than pollen grain size itself, certainly among species with dry stigmas; and it might even emerge that larger grains have proportionately greater apertural areas through which intine contact might be made. The rationale of this is simply that, wherever hydration depends upon the establishment of continuous water films between the apertural intine and a dry stigma surface, selection might be expected to favor a geometry that would optimize the chance of direct contact between the aperture and the porous cuticle of the papilla.

Aperture shape becomes a factor here, and also the porosity of the exine. Only some 0.4% of the large pollen grains of *Zea mays* might, on statistical grounds, be expected to hydrate at all from the stigma were the only pathway of

water uptake the single aperture; as it is, most grains hydrate through the micropores of the exine. In contrast, the equally large pollen grains of certain Malvaceae have a thick nexine, unperforated by micropores. Compensation is here offered by the presence of numerous apertures, and sometimes also by another intriguing adaptation, this time on the part of the stigma papillae, which often embrace the grains after capture, so bringing the porous papillar cuticle into contact with several apertures.

Stigmas of the dry type do offer rather difficult conditions for pollen hydration, and adjustment to these must obviously require specialization of the exine and the apertural mechanisms. The evolution of forate exines, with several more or less circular apertures, seems to be one of these specializations. I am aware of no exceptions to the rule that forate exines are associated with dry stigmas; and this certainly seems to imply a physiological correlation. The clue is given by the behavior of pollens like those of the Caryophyllaceae on the stigma (Heslop-Harrison et al., 1975). The apertures closest to the stigma surface form the first routes for the ingress of water, and the intine underlying these apertures is thus the first to become hydrated. Presumably, enzymic softening of the intine begins rather earlier at these sites, so it is to be expected that the tube should emerge from one of them, as is generally to be observed. In the large grains of genera like *Hibiscus* the same localization of emission and tube outgrowth can be seen, and the Malvaceae also offer spectacular examples of polysiphonious germination where tubes emerge from several of the apertures adjacent to the contact face with the stigma.

The adaptive value of the forate exine therefore probably lies in the way it allows for the highly localized activation of apertural intine sites. The adaptation would clearly have no value on a wet stigma where hydration and germination occur while the grain is wholly or partly immersed in the stigma secretions.

Anomalous Pollen Types and the Transfer of Function

In my introductory remarks I referred to the likelihood that the diversity of pollen walls would not only reflect the multiplicity of selective pressures to which the various functions have been exposed in the course of angiosperm evolution, but also the fact that the same functional end may well be attained by different means. It is an enlightening exercise to compare different taxonomic alliances with these points in mind. There are clearly certain irreducible functions, such for example as those concerned with hydration, tube emergence, and stigma penetration. There are others that are of the nature of options. To take one instance: in some families, as we have seen, the exine is the repository for a substantial load of sporophytically synthesized materials, which apparently have functions in the interaction with the stigma; yet in other families, the exine carries little or no tapetal material.

This reduction in the function of the exine is strikingly obvious in certain groups. The baculate type of exine has been lost from various modern angiosperms, apparently by secondary simplification (Nilsson & Skvarla, 1969), and this must of course have meant the sacrifice of the capacity to convey sporophytic surface materials in exine cavities. A sequence can be traced in the group of monocotyledonous families constituting Engler's Scitamineae, including Musa-

ceae, Strelitziaceae, Heliconiaceae, Marantaceae, Cannaceae, and Zingiberaceae (Heslop-Harrison, 1976). In some representatives, an exine is present with a rudimentary baculate system; in others it forms no more than a single thin layer without included cavities and in still others (e.g., *Canna*, Skvarla & Rowley, 1970) it is reduced to isolated papillae overlying a thick intine. This sequence can be connected through various Liliiflorae such as *Crocus* and *Sternbergia* to genera in which the baculate exine is fully developed. Intriguingly, this regression of the exine is accompanied by an elaboration of the intine. The intine is generally thicker in the genera with reduced exines, and the system of internal inclusions is better developed, in the extreme examples extending over the whole surface of the grain, as first reported by Skvarla & Rowley (1970). These inclusions are derived from microvilluslike extensions of the membrane of the vegetative cell which are cut off into the intine as it thickens, and each is a repository of a range of hydrolytic enzymes similar to those found at the apertural sites of aperturate pollens (Heslop-Harrison, 1975b, Y. Heslop-Harrison, 1977). In some sense, therefore, we see a function of the exine transferred to the intine; but the detailed physiological relationships remain to be worked out. It would, for example, be profitable now to compare the water economy of the pollens of *Lilium* and the exineless Scitamineae. The absence of the protecting exine in the latter must have some consequence; but I suspect it might prove that a full understanding of the situation would require that the pollination biology be taken into account, and especially the conditions of dispersal in the natural habitat.

LITERATURE CITED

HESLOP-HARRISON, J. 1968. Tapetal origin of pollen coat substances in *Lilium*. New Phytol. 67: 779–786.

———. 1971. Sporopollenin in the biological context. Pp. 1–30, *in* J. Brooks, P. R. Grant, M. D. Muir, P. van Gijzel & G. Shaw (editors), Sporopollenin. Academic Press, London.

———. 1975a. Incompatibility and the pollen-stigma interaction. Annual Rev. Pl. Physiol. 26: 403–425.

———. 1975b. The physiology of the pollen grain surface. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 190: 275–299.

———. 1976. The adaptive significance of the exine. *In* I. K. Ferguson & J. Muller (editors), The Evolutionary Significance of the Exine. Linn. Soc. Symp. Ser. 1: 27–38.

———. 1978. Genetics and physiology of angiosperm incompatibility systems. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 202: 73–92.

———. 1979. An interpretation of the hydrodynamics of pollen. Amer. J. Bot. 66: 737–743.

———, Y. HESLOP-HARRISON & J. BARBER. 1975. The stigma surface in incompatibility responses. Proc. Roy. Soc. London, Ser. B, Biol. Sci. 188: 287–297.

———, ———, R. B. KNOX & B. HOWLETT. 1973. Pollen-wall proteins: "gametophytic" and "sporophytic" fractions in the pollen walls of Malvaceae. Ann. Bot. (London), n.s., 37: 403–412.

———, R. B. KNOX, Y. HESLOP-HARRISON & O. MATTSSON. 1975. Pollen-wall proteins: emission and role in incompatibility responses. *In* J. G. Duckett & P. A. Racey (editors), The Biology of the Male Gamete. Biol. J. Linn. Soc. Suppl. 1: 189–202.

HESLOP-HARRISON, Y. 1977. The pollen-stigma interaction: pollen tube penetration in *Crocus*. Ann. Bot. (London), n.s., 41: 913–922.

———, & K. R. SHIVANNA. 1977. The receptive surface of the angiosperm stigma. Ann. Bot. (London), n.s., 41: 1233–1258.

HOWLETT, B. J., R. B. KNOX & J. HESLOP-HARRISON. 1973. Pollen-wall proteins: release of the allergen Antigen E from intine and exine sites in pollen grains of ragweed and *Cosmos*. J. Cell Sci. 13: 603–619.

KERNER, A. J. & F. W. OLIVER. 1904. The Natural History of Plants. Gresham, London.

KONAR, R. N. & H. F. LINSKENS. 1966. Physiology and biochemistry of the stigmatic fluid of *Petunia hybrida* L. Planta 71: 372–387.

——— & R. G. STANLEY. 1969. Wall-softening enzymes in the gynoecium and pollen of *Hemerocallis fulva*. Planta 84: 304–310.

NILSSON, S. & J. J. SKVARLA. 1969. Pollen morphology of saprophytic taxa in the Gentianaceae. Ann. Missouri Bot. Gard. 56: 420–438.

LINSKENS, H. F. 1967. Pollen. *In* H. F. Linskens (editor), Handbuch der Pflanzenphysiologie. Vol. 18: 368–406. Springer-Verlag, Berlin.

PAYNE, W. W. 1972. Observations of harmomegathy in pollen of Anthophyta. Grana 12: 93–98.

ROLAND, F. 1966. Étude de l'ultrastructure des apertures: pollens à pores. Pollen & Spores 8: 409–419.

———. 1968. Étude de l'ultrastructure des apertures: II. Pollens à sillons. Pollen & Spores 10: 479–519.

ROWLEY, J. R. 1960. The exine structure of "cereal" and "wild" type grass pollen. Grana Palynol. 2: 9–15.

SITTE, P. 1953. Untersuchungen zur submikroskopischen Morphologie der Pollen und Sporenembranen. Mikroskopie 8: 290–299.

SKVARLA, J. J. & J. ROWLEY. 1970. The pollen wall of *Canna* and its similarity to the germinal apertures of other pollen. Amer. J. Bot. 57: 519–529.

STANLEY, R. G. & H. F. LINSKENS. 1974. Pollen: Biology, Chemistry and Management. Springer-Verlag, Berlin.

VON MOHL, H. 1834. Sur la structure et les formes des grains de pollen. Ann. Sci. Nat. Bot., sér. 2, 3: 148–180, 220–236, 304–346.

WATANABE, K. 1955. Studies on the germination of grass pollen. 1. Liquid exudation of the pollen on the stigma before germination. Bot. Mag. (Tokyo) 68: 40–44.

WODEHOUSE, R. P. 1935. Pollen Grains. McGraw-Hill, New York.